Microbial control of the cotton leafworm by bacteria and its preparation

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Laboratory experiments were carried out to estimate the efficacy of three bacterial commercial insecticides of Bacillus thuringiensis (Berliner) on each of the six larval instars of Spodoptera littoralis (Bosid .) and the survivors of subsequent stages . Recorded data included mortalities , malformations , egg — laying activity of resultant adults and hatchability percentages among the deposited eggs . Studies extended also to evaluate the efficacy of Dipel 2X (which showed best results) when sprayed on cotton plants artificially infested with the egg — masses . Also , trials were made to determine the role of each of 3 materials used as photoprotectants for B. t. kurstaki from the detrimental effect of U.V. (in the laboratory) and the effect of UV emmited through sunlight(in the field) . Studies included also estimations for the efficacy of a new bioinsecticide (MVP n.) , depending on B. t. kurstaki S endototin bioencapsulated in killed Pseudomonas flouresens , on S. littoralis 1st and 3rd instar larvae and the survivors , and also the effect of UV (in laboratory and field) on the biological activity of this product against S. littoralis larvae . Studies covered also the effect of Dipel 2X treatments by LC20 and LC50 to S. littoralis 2nd and 4th instar larvae on the larval haemolymph and also the effect of the same product at LC50 and LC90 to 1St and 3rd instar larvae of S. littoralis on the histology of mid — gut .The obtained results may be summarized as follows :— Effect on different larval instars of S littoralis:1 -1- Effect on larval mortality :By two days feeding of different of each of the six S. littoralis larval instars on leaves treated with different concentrations of Dipel 2X195, Dipel EC and Ecotech , the following percentages were recorded among treated larvae :•Newly hatched larvae ; 26.7 - 100 , 26.7 -100 and 26.7 - 100 % .•24 h. old 1st larval instar ; 23.3 - 96.7, 26.7 - 96.7 and 26.7 - 93.3%.•2nd larval instar ; 20.0 - 100 ,26.7- 100 and 26.7 - 93.3 % .•3rd larval instar ; 26.7 - 96.7 ,20- 96.7 and 20.0 - 90.0 % .•4th larval instar ; 26.7 - 96.7, 13.3- 96.7 and 13.3 - 100 % .•5th larval instar ; 13.3 - 93.3, 13.3- 90.0 and 16.7 - 93.3 % .•6th larval instar ; 10.0 - 90.0, 6.7- 86.7 and 10.0 - 90.0 % .After 5 days of starting treatment by Dipel 2X , Dipel EC and Ecotech , the LC 50’s were 0.464 x 103, 0.899 x 103 and 0.810 x 103 I. U. / mg for treatment of newly hatched larvae ; 0.998 x 103 , 1.266 and 1.188 x 103 I. U. / mg for 24 h. old 1st instar larvae , 1.06 x 103, 1.528 x 103 and 1.352 x 103 I.U./ mg for 2nd instar larvae , 1.622 x 103, 2.11 x 103 and 2.077 x 1031. U./ mg for 3rd instar larvae , 1.843 x 103 , 2.267 x 103 and 3.654 x 103 I. U./ mg for 4th instar larvae , 4.144 x 103, 4.691 x 103 and 4694 x 1031. U. / mg for 5th instar larvae 4.288 x 103, 6.133 x 103 and5.409 x 103 I. U. /mg for the 6th instar larvae fed on castor - bean leaves treated by Dipel 2X , Dipel EC and Ecotech , respectively .1.2. Effect of larval feeding on castor - bean leaves treated by the LC 50’ s on the survivors :By different instar larval feeding on LC50 of Dipel 2X , Dipel EC and Ecotech , the following biological data were obtained :•Malformations among pupae & subsequent moths :196Newly hatched larvae ; 13.64 & 15.0 ,8.33 & 18.18 and 10.25 &8.57 % .< 1st larval instar ; 21.57&17.65,15.91 &16.22 and 13.15&15.15%2nd larval instar ;13.16 &27.2, 18.96 & 25.0 and 11.43 &16.13% .< 3rd larval instar ; 11.11 &25 , 18.92 & 22.13 and 11.76 &13.33% .4th larval instar ; 25.0&12.5 , 17.14& 10.34 and 14.28&13.33% .5th larval instar ; 12.9&14.28 , 6.68 & 22.22 and 16.67 &14.28.< 6th larval instal ;13.33&19.23 , 10.18 & 18.18 and 8.0 &16.67% .These percentages of malformations were obtained among the developing pupae & moths after treatments by the LC50 of Dipel 2X , Dipel EC and Ecotech , respectively .Each of the six larval instars of S. littoralis was fed for 48 h. on castor — bean leaves treated with Dipel 2X ,

Dipel EC and Ecotech at the LC50 , then on untreated leaves . The following biological data were recorded on the larvae and subsequent stages .•Larval and pupal period :Treatment of all larval instars caused significant prolongations in the larval periods , but the differences than control were more pronounced by larval treatments at earlier instarsThe pupal durations of those from treated larvae were also longer than that recorded from the control pupae , but the differences were significant by treatments of newly hatched larvae and those of 24 h. 1st , 2nd , 3rd and 4th instars t , but insignificant than control among those developed from the 5th and 6th instars .•Adults' longevity :The life spans of males and females were found to be reduced due to treatment of either of the six larval instars by the LC50 of Dipel2X , Dipel EC or Ecotech . These periods averaged 4.2 — 6.2 for males and 4.6 — 6.6 days for females from treated larvae , opposed to 8.6 — 8.8 and 9 — 9.4 days for control males and females , respectively .•Oviposition period :This period was also reduced significantly due to larval treatment . The obtained averages of this period ranged from 4.9 — 7.1 days , while it averaged 8.3 — 9 days for the control females .•Eggs' reproductivity :The resultant females showed , significant , reductions in their total numbers of eggs laid / female , due to feeding of different larval instars on castor — bean leaves treated with either of the three bioinsecticides . The obtained values for moth females from larval treatments by the LC50 of Dipel 2X , Dipel EC , Ecotech and control were , respectively , as follows :<Newly hatched larvae ; 136.2 , 143 , 156.6 and 514 egg / female .•1st larval instar ; 144.2 , 151.8 ,166.6 and 514 egg / female .<2nd larval instar ; 178.6 , 179 , 199.7 and 526.2 egg / female .•3rd larval instar ; 221 , 128.8 , 295 and 534.3 egg / female .<4th larva instar ; 283.8 , 287 , 297 and 540 egg / female .•5th larval instar ; 368 , 380 , 390 and 530 egg / female .•6th larval instar ; 400 , 410 , 440 and 540.6 egg / female .2. Field studies (Artificial infestation) :Dipel 2X was applied , in the field ,on August , 4th , 1998 , at the recommended rate (200 gm / 400L / water / feddan) on cotton plants that were artificially infested by the cotton leafworm egg — masses . Spraying took place just after distribution of the eggs in the field .198Random samples , of 75 cotton leaves each / plot were collected 7 , 10 and 15 days after spraying .Data indicated that spraying of Dipel 2X caused reductions in the rate of infestation to cotton leaves by the cotton leafworm by 51.0 , 51.01 , 56.31 and 56.56 % than control after 7 , 10 and 15 days , of treatment , respectively .3. Materials used for encapsulating B. thuringiensis spores against ultraviolet :In order to reduce the well known detrimental effect of ultraviolet emmited through the sunlight , three different materials (Shellac , melanin and neste — coffee) were used for encapsulating B. t. kurstaki spores in Dipel 2X assayed in the laboratory and in the field to find out their role as photoprotectants able to absorb the ultraviolet rays .• In the laboratory :Dipel 2X at 5 % concentration , that mixed with flour + yeast and that mixed with flour + yeast + either of the three photoprotectant materials were exposed to U.V. rays for 4 , 8 , 12 and 16 hours , than assayed against S. littoralis 1st instar larvae By using the bioinsecticide alone or the mixtures before exposure to UV , the mortality percentages among treated larvae ranged between 65 — 67 % . While , by exposure to UV , all the assayed materials showed different rates of reduction in mortality % . The severest effect of UV on the activity of B. t. kurstaki occurred when the bioinsecticide was exposed to UV without any additives (mortality percentages ; 35 % by exposure for 4 hours and only 20 % by exposure for 16 hours) . By adding flour and yeast to the bioinsecticides , the mortality percentages were 47 and 30 % , respectively . But these percentageswere found to be raised by adding shellac , melanin or neste — coffee to be 64 & 61 , 64 & 62 and 62 & 57 % after 4 & 16 hours exposure to UV irradion , respectively . Thus confirming the excellent effect of the three materials as photoprotectants and consequently preserving the bioactivity of B. t. kurstaki spores , as the original activity remaining (OAR) remained high by using the three materials , being 93.85 , 95.38 and 87.69 , respectively whent the mixture were exposed to UV for 16 hours , opposed to only 37.77 and 46.15 for the bioinsecticide and mixed with flour and yeast .• In the field :Dipel 2X and the aforementioned mixtures were on cotton plants in the field and the treated leaves were offered to S. littoralis 1St instar larvae after different period from treatment . The freshly sprayed leaves caused 64 — 66 % mortality among treated larva . By exposure to field conditions the viability of B. t. kurstaki spores were found to be reduced greatly by exposure to field conditions , and the effect of these conditions increased as the exposure period prolonged . The effect was drastic when Dipel 2X was used alone (16 and 10 % mortality after 4 and 5 days from spraying , respectively) . Adding

shellac , melanin or neste — coffee to Dipel 2X kept , to great extent , the viability of B. t. kurstaki spores in the field as the mortality percentages of S. littoralis larvae after 4 and 5 days were 3 to more than 4 times that occurred by Dipel 2X alone (50 , 46 & 40 after 4 days and 45 , 42 & 35 after 5 days by using the three materials , respectively) .The protection activity of shellac , melanin or neste — coffee on B. t. kurstaki after field spraying was clear also when some biological parameters were recorded on the surviving individuals of S.littoralis after treatment . That was clear on the prepupal and pupal mortalities which were greatly reduced after 3 days of Dipel 2X application in the field , but remained higher when either of the three materials was added . Also the larval and pupal durations remained , significantly , longer than control by using the mixtures of Dipel 2X + flour + yeast + either of shellac , melanin or neste — coffee . Three days after field spraying of Dipel 2X alone , the loss of B. t. kurstaki activity was clear when the fecundity of resultant moths were determined as the oviposition period and eggs reproductivity (10 days and 515 eggs / y) were nearly the same as that of control adults (10.3 days and 556 eggs ty) . While in case of mixtures containing shellac , melanin or neste — coffee the bioactivity of the bioinsecticide remained even after 3 days of spraying (oviposition periods 6.3 , 7.3 and 7.7 days and eggs reproductivity 301 , 320 and 331 eggs / y , respectively) .The obtained laboratory and field data confirmed the excellent effect of the assayed materials (shellac , melanin and neste — coffee) as photoprotectants B. thuringiensis of the bioinsecticide .4 — Assays on MVPII , a new bioinsecticide of B. t. kurstaki endotoxin :4-1- Effect on S. littoralis larval mortality :The efficacy of MVPII on S. littoralis 1st and 3rd instar was assayed in the laboratory by 2 days larval feeding on castor — bean leaf discs treated with different concentrations of the product followed by 3 days feeding on fresh untreated leaves . Larval mortalities ranged from 13.3 —96.7 % by treatment of 1st instar and 11 — 93.3 by treatment of 3rd instar . The effect was a concentration dependent , The LC50’s were 0.039 and0.141 ml for the two larval instar treatments , respectively indicating lower susceptibility of the elder larvae .4-2- Biological effect on survivors after treatment :After 2 days treatment of S. littoralis 1St and 3rd larval instars by the LC50 of MVPII , the survivors showed , significantly , longer larval and pupal periods ; 7.25 & 9.52 % malformations among pupae and18.75 & 15.79 % malformations among adults from treated 1St and 3rd instar larvae , respectively ; shorter oviposition periods ; shorter life —span of adults and decreased eggs productivity (318 & 338 eggs / respectively opposed to 594 eggs / a control female) .4-3- Effect of UV on the efficacy of MVPIT:4-3-a In laboratory :mvpn was exposed to the UV source for different periods (16 , 36 , 44 and 56 hours) then compared with non — irradiated material , against the 1” larval instar of S. littoralis . The mortality percentages were 93.3 , 76.6 , 70.0 , 46.7 % respectively , opposed to 96.6 % from the nonirradiated material .4-3-b- In the field :Three castor — bean plants were sprayed with MVPII at concentration 0.3 ml / 100 ml water . Treated leaves were collected just after spraying and after 2 , 4 , 9 , 11 and 14 days from treatment to be offered to S. littoralis instar larvae . After 5 days from feeding on treated food , the percentage mortality were 86.7 , 80.0 , 60.0 , 50 , and 36.7 % mentioned periods , respectively , opposed to 93.3 % from treatment at zero time .5 - Haemolymph studies :5-1-Effect of Dipel 2X treatment on total haemocyte counts (THCs) :The total haemocyte counts in the haemolymph of untreated S. littoralis larva was 30583 cells / mm3 . After 5 days of 2nd and 4th instar larval treatment with LC20 and LC50 of Dipel 2X , THCs were found to be reduced significantly to 21566 and 15316 cells / mm3 in the former treatments , and to 20733 and 18550 cells / mm3 in the latter one , for the two instars , respectively .5-2-Effect of larval treatment on differential haemocyte counts (DHCs) :The haemolymph inspections included the dimensions cell and nuclei from each of the eight haemocyte types (length and width) in 2nd and 4th instar larvae treated with Dipel 2X at the LC20 and LC50 levels as well as in healthy larvae of the same age . The nucleus cell ratio was also estimated for each treatment and also for the control after 72 , 96 and 120 hours of treatment . Due to larval treatment , the cell and nucleus dimensions , clearly , decreased than control , in addition to irrigularity of cell outlines after treatment . Also some increases in the cells and nuclei’s dimensions were detected . In many haemocyte types , the nuclei lost their central position and moved towards the cell walls due to Dipel 2X treatment .5-3- Effect of Dipel 2X treatments on quantitative analysis of haemocytes :The quantitative analysis of different haemocyte types were estimated on 2nd and 4th instar S. littoralis larvae fed on Dipel 2X . Generally , the prohaemocytes occupied more than 50% of

the totalhaemocyte counts , followed by the plasmatocytes , granulated cells and spindle cells ,while , the remaining four haemocyte types were much fewer in quantity .Larval feeding on leaves treated with Dipel 2X at LC20 or LCso resulted in reductions in the number of prohaemocytes , oenocytoides , spherule cells and cystocytes than control . While on the controry increases occurred in the percentages of phagocytic cells (Plasmatocytes , spindle and granulated cells) . This normal as these phagocytes play an active defense role against any strange material . the adipohaemocyte counts also increased due to larval treatment with Dipel 2X .6-Histologic) studies :S. littoralis 1st and 3rd instar larvae were fed for 48 hours on castor —bean leaves contaminated with LC20 and LC 50 of Dipel 2X and MVPII . Tested larvae were , then transferred to feed on clean untreated leaves . Surviving larvae after 24 , 48 , 72 ,96 and 120 hours from treatment were histologically studied to detect the effect of the bioibsecticide treatment on S. littoralis mid gut tissues . The histobathologicl effect cased by Dipel 2X on the mid gut of lst and 3rd instar larvae involve separation of epithelial cells from the basement membrane , and in some case the basement membrane disappered , and in some cases , the epithelial cells appeared deformed and became of smaller size than size those of control . On the other hand , the MVPII, preparation induced rapid effect on the mid gut epithelium , when the feeding of S. littoralis larvae with MVPII led to leakaged of the cytoplasm contents from the damaged cells into the gut lumen and binding of the toxins to the peritrophic membrane .